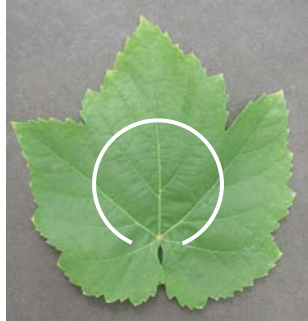


Progress in Genetic Options for Suppression of Pierce's Disease in Grape

David Gilchrist, James Lincoln, Barney Ward, Douglas Cook

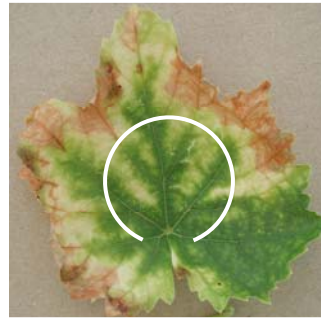


Healthy or Disease Resistant



The Goal is to Avoid PD Symptoms
by
Suppression of Inherent Susceptibility
through
Genetic Intervention

Xylella Triggered
Programmed Cell Death



PD symptoms on susceptible grape:
23/25 petiole confocal microscope fields
were positive for GFP-*Xylella fastidiosa*
but none found outside the white arc.

Dying cells exhibit features of PCD with
apoptotic markers

SUPPRESSION OF INHERENT SUSCEPTIBILITY TRIGGERED IN GRAPE BY AN ENDOPHYTE GONE BAD

Genetic modification of plants to prevent
expression of PD susceptibility in grape



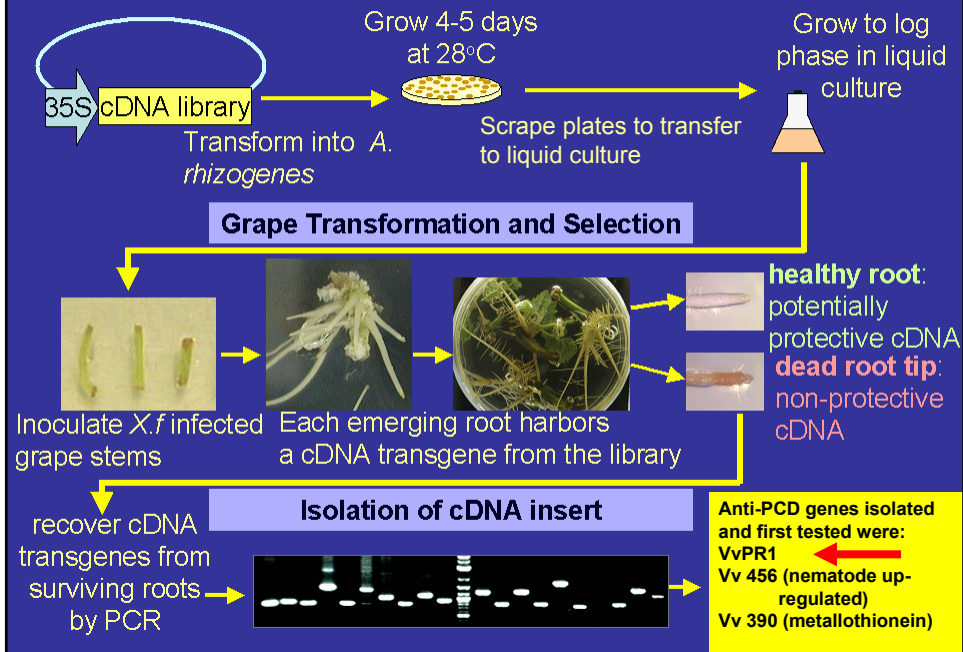
Programmed cell death



“Return of the Endophyte”

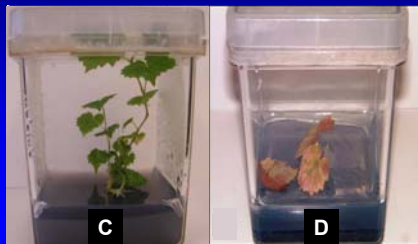
Suppress, either transiently or permanently, the cellular events
that lead to susceptibility, manifested as cell death, when few or
no cells have been compromised but have been stimulated

Agrobacterium rhizogenes screen and assay for protective transgenes



EFFECT OF THE PR 1 ANTI-PCD GENE IN MICRO-PROPAGATED TRANSGENIC PLANTS WHEN PROGRAMMED CELL DEATH IS ACTIVATED

The symptoms were blocked in grape plants expressing the PR1/P14 transgene in box (C), which remained alive 6 months after induction of symptoms, while plant death via PCD occurred in the untransformed plant (D)



History of the PR1 Gene in Plant Response to Pathogens

- The earliest pathogenesis-related protein identified; more than two decades ago
- The most widely used marker of genetic resistance response in plants
- **The only pathogenesis-related protein without a known function**
- The only pathogenesis-related protein that may be translationally regulated
- mRNA transcript induced in both animal and plant disease situations where programmed cell death (apoptosis) appears to play a determining role.

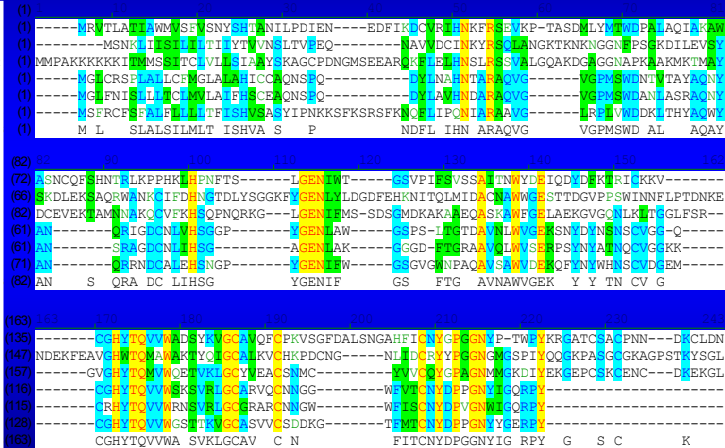
PR1 FAMILY PROTEIN ALINGMENT

Evidence for global role in regulating cell death
in diseases associated with plants and animals

Human brain tumor GliPR1
Meloidogyne incognita MSP1
Human hookworm saliva ASP2
Vitis vinifera VVPR1
Lycopersicon esculentum P14
Medicago truncatula PR1

Human brain tumor GliPR1
Meloidogyne incognita MSP1
Human hookworm saliva ASP2
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Lycopersicon esculentum P14
Medicago truncatula PR1

Human brain tumor GliPR1
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Human hookworm saliva ASP2
Vitis vinifera VVPR1
Lycopersicon esculentum P14
Medicago truncatula PR1



Based on ClustalW algorithm
Vector NTI

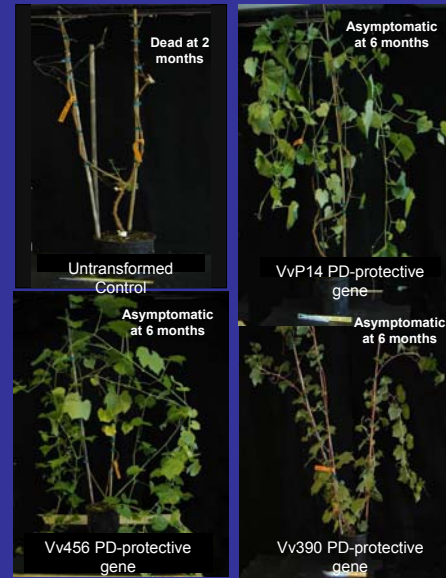
5 POINT RATING SCALE REFLECTING THE SEQUENCE OF DEATH IN
UNTRANSFORMED CONTROL PLANTS AND THE RESPONSE SPECTRUM BY
GRAPE PLANTS EXPRESSING THE ANTI-PCD TRANSGENES TO INOCULATION
WITH *X. fastidiosa*, COMPARED WITH AN UNINOCULATED CONTROL PLANT



Table 1. VARIETY-Freedom: INITIAL LINES TRANSFORMED AND ANALYZED

Genotype	# of Independent Transformants	# of Plants
FR - P14LD	16 (5 tested to date)	293
FR - Y-456	7 (2 tested to date)	112
FR - 390	9 (3 tested to date)	126
FR - GFP control	10 (5 tested to date)	140
FR - Untransformed	10 tested in this assay	NA
Total	32	671

Table 2. Plant name	Relevant genotype	Disease Category 4-5 plants similar to images in Figure 1 at 6 months post inoculation	Range of bacterial load per gm of stem in asymptomatic category 5 branch at 6 months post inoculation
CBP14-14	CaMV 35S-driven PR1	90%	10^3
CBP14-13	CaMV 35S-driven PR1	80%	$10^4 - 10^5$
CBP14-11	CaMV 35S-driven PR1	75%	10^4
CB456-3	CaMV 35S-driven "nematode up- regulated" gene	90%	$10^4 - 10^5$
CB456-6	CaMV 35S-driven "nematode up- regulated" gene	85%	$10^4 - 10^5$
CB390-8	CaMV 35S-driven metallothionein	75%	$10^4 - 10^5$
CBGFP	CaMV 35S-driven GFP transformed and untransformed plants as controls	All dead at 3 months	$\sim 10^8$ at the time the plants began to die at 2 months post inoculation
<i>Vitis californica</i>	Asymptomatic wild type untransformed grape host	no death after 12 months post inoculation	10^4



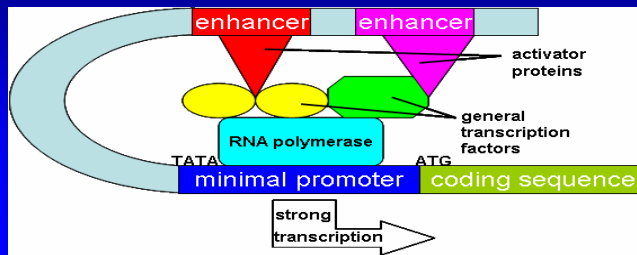
Representative untransformed control and transgenic plants expressing the genes indicated in tables 1 & 2. All plants photographed 6 months after inoculation with *Xylella fastidiosa*; control plants 100% dead at 2 months

Summary



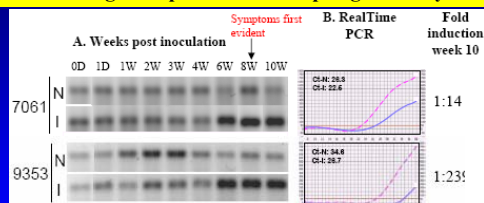
- Programmed cell death pathway activation leads to foliar, cane and root death in Pierce's Disease
- Functional screens of cDNA libraries of grape and tomato identified several potential anti-PCD plant genes
- Expression of anti-PCD genes in infected grape plants suppresses symptoms of PD and limits bacterial titer
- PR1 protein normally secreted outside the cell with the potential for moving in the vascular stream

THE SEARCH FOR *Xylella fastidiosa* INDUCIBLE PROMOTERS



- TO TEST OR EXPRESS PUTATIVE GENES CAPABLE OF BLOCKING INHERENT SUSCEPTIBILITY AND
- TO EXPRESS THEM ONLY AT SITES AND TIMES WHEN BACTERIAL-DERIVED SIGNALS ARE PRESENT
- MAKE AVAILABLE TO OTHER RESEARCHERS TO ENABLE THE ACTIVATION OF *Xylella*-RESPONSIVE GENES IN THE ROOT STOCK OR SCION FOR TESTING ANTIBIOTIC ACTIVITY, MOBILE RNA-MEDIATED PROTECTION, ETC.

Xylella fastidiosa responsive promoters were obtained from genes found to be up-regulated in *X. fastidiosa* infected field grown plants but not up-regulated by water stress alone

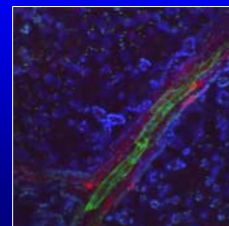
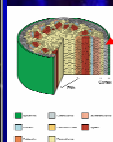


Expression of promoter G7061 in plant #11 fused to GFP detected after 7 days in petiole of leaves attached to a detached branch into which *Xylella fastidiosa* was taken up through the cut end of the branch at 10 ml of 10^7 cfu/ml. Water control shows no promoter activation.

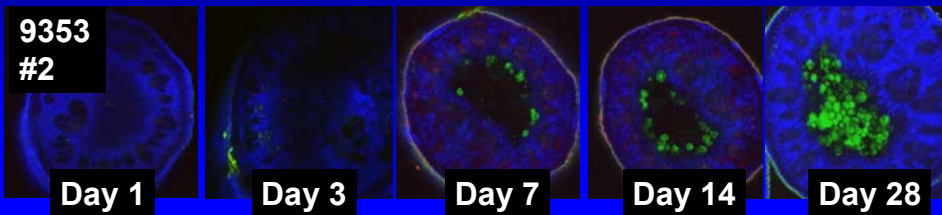
Water uptake



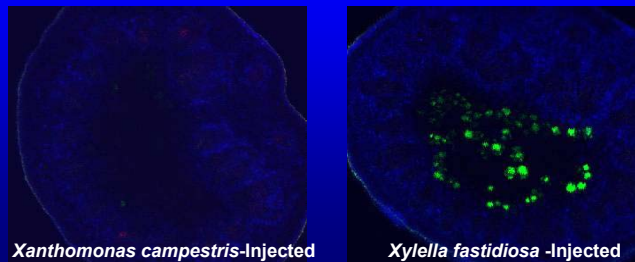
Xf uptake



Time course analysis of the expression of promoter G9353 fused to GFP in response to the presence of *X. fastidiosa* in the vascular system of grape



Specificity of the response of promoter G9353 fused to GFP in response to the presence of *X. fastidiosa* in the vascular system of grape



Summary



- *X. fastidiosa*-induced promoters have been isolated and confirmed to drive expression of the GFP fused to the putative promoters in *Xf*-infected tissue but not in tissues inoculated with *Xanthomonas campestris* pv. *vesicatoria*
- These expression cassettes will allow regulated gene expression in particular tissues (cells surrounding the vascular tissue) and/or in response to particular situations (e.g., sharpshooter feeding or *Xylella* presence).
- The cassettes can provide the opportunity for time- and site- specific analysis of signal exchange between insect/plant or bacteria/plant interactions
- Cassettes enable direct secretion of transgenic proteins or small RNAs to the apoplastic compartments surrounding cells where the pathogen resides if requisite leader sequences are integrated